SUPPLEMENTARY MATERIAL

A bioactive new protostane-type triterpenoid from *Alisma plantago-aquatica* subsp. *orientale* (Sam.) Sam.

Ya-Li Wang[1,2,+], Jia-Hao Liang[2,+], Jian-Chao Zhao[1,2,+], Xiang-Ge Tian[2], Xiao-Kui Huo[2], Lei Feng[2], Jing Ning[2], Chao Wang[2], Bao-Jing Zhang[2], Gang Chen[1,*], Ning Li[1,*] and Cheng-Peng Sun[2,*]

[1] *Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Department of Natural Products Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China*

[2] *College of Pharmacy, College (Institute) of Integrative Medicine, Dalian Medical University, Dalian, China.*

[+]*These authors contributed equally to this work.*

Abstract: A new protostane-type triterpenoid, 5β,29-dihydroxy alisol A (**1**) was isolated from *Alisma plantago-aquatica* subsp. *orientale* (Sam.) Sam. as well as 12-deoxyphorbol-13α-pentadecanoate (**2**). We first report the presence of compound **2** in the genus *Alisma*. Their structures were established on the basis of 1D and 2D NMR, and HRESIMS spectroscopic analyses. All the isolated compounds were assayed for their inhibitory effects against human carboxylesterase 2 (HCE-2). Compounds **1** and **2** displayed inhibitory activities against HCE-2 with IC_{50} values of 29.2 and 4.6 μM, respectively. The interaction mechanisms of HCE-2 with compounds **1** and **2** were investigated by molecular docking, respectively.

Keywords: *Alisma orientale*; protostane; HCE-2 inhibitor; molecular docking

List of supporting information

Table S1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of compound **1** in MeOH-d_4

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	32.4	2.26 m	16	30.5	2.26 m
		2.16 m			2.19 m
2	34.8	2.82 m	17	137.0	
		2.22 m	18	24.7	1.04 s
3	223.7		19	26.5	1.11 s
4	52.6		20	30.0	2.83 m
5	84.5		21	20.9	1.03 d (6.8)
6	20.6	1.68 m	22	42.1	1.59 m
		1.36 m			1.48 m
7	35.8	2.05 m	23	70.5	3.73 m
		1.27 m	24	79.7	3.02 d (1.6)
8	41.7		25	74.8	
9	51.1	1.79 d (11.1)	26	27.3	1.21 s
10	38.1		27	26.7	1.20 s
11	70.7	3.81 m	28	23.7	1.24 s
12	35.2	2.79 m	29	66.2	4.00 d (11.6)
		2.05 m			3.49 d (11.6)
13	139.2		30	23.7	1.17 s
14	58.4				
15	31.8	1.93 m			
		1.33 m			

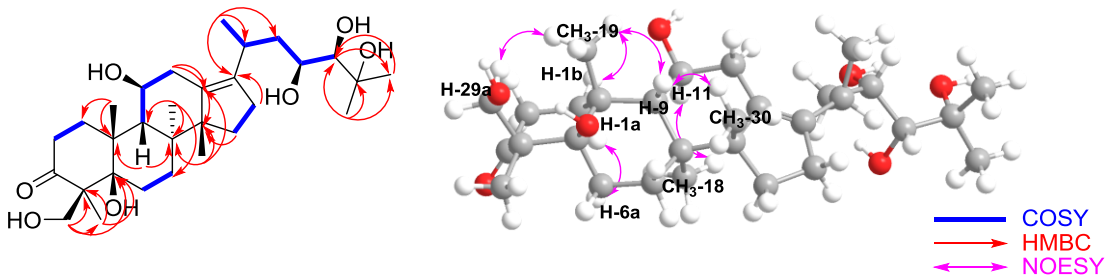

Figure S1. Selected COSY, HMBC, and NOESY correlations of compound **1**

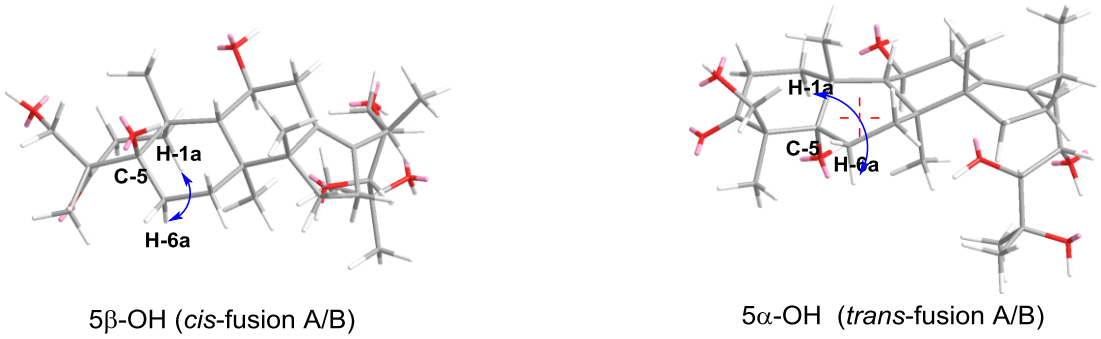

Figure S2. Two possible configurations of compound **1** and key NOESY correlation of H-1a with H-6a

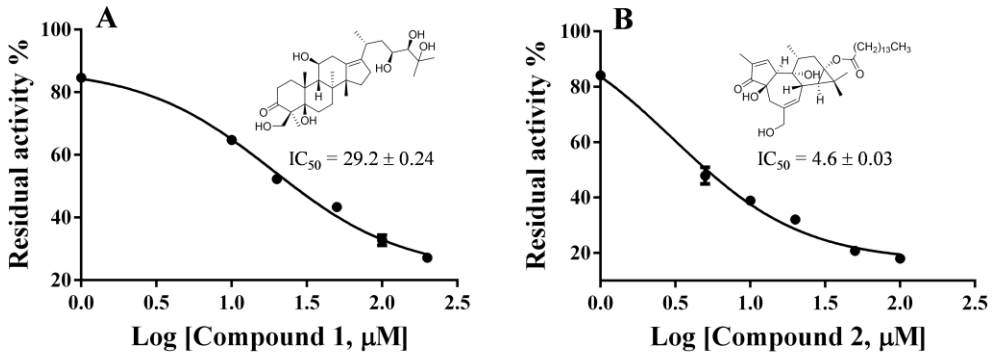

Figure S3. Inhibitory HCE-2 effects of compounds **1** and **2**

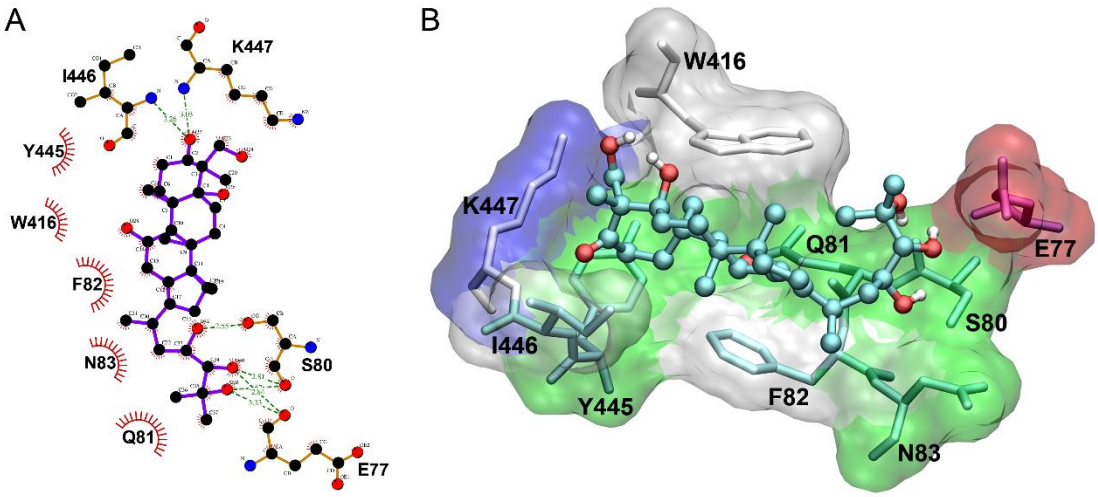

Figure S4. 2D (A) and 3D (B) structure of compound **1** with HCE-2.

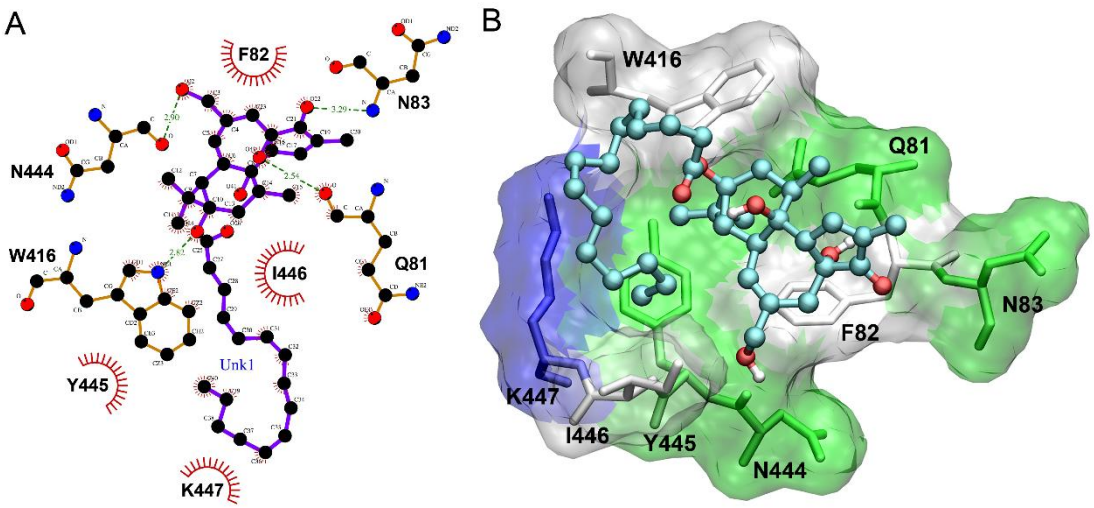

Figure S5. 2D (A) and 3D (B) structure of compound **2** with HCE-2.

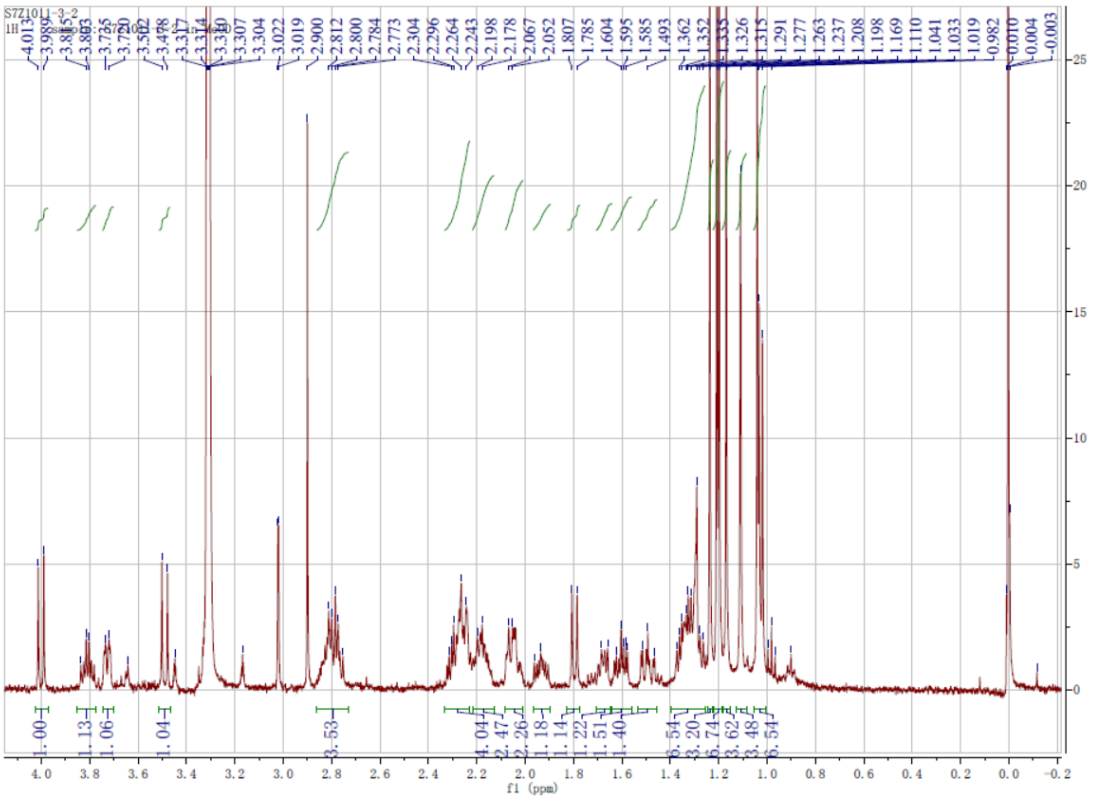

Figure S6. ^{1}H-NMR spectrum of **1** (600 MHz, MeOH-d_4)

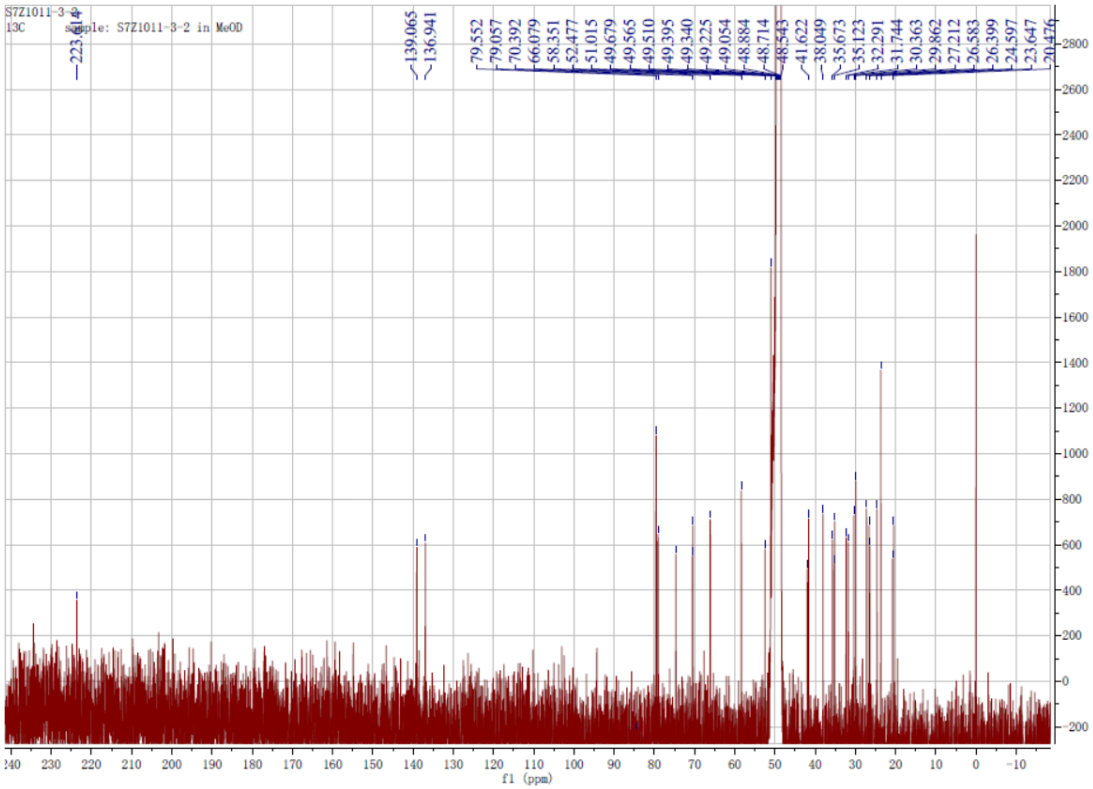

Figure S7. ^{13}C-NMR spectrum of **1** (150 MHz, MeOH-d_4)

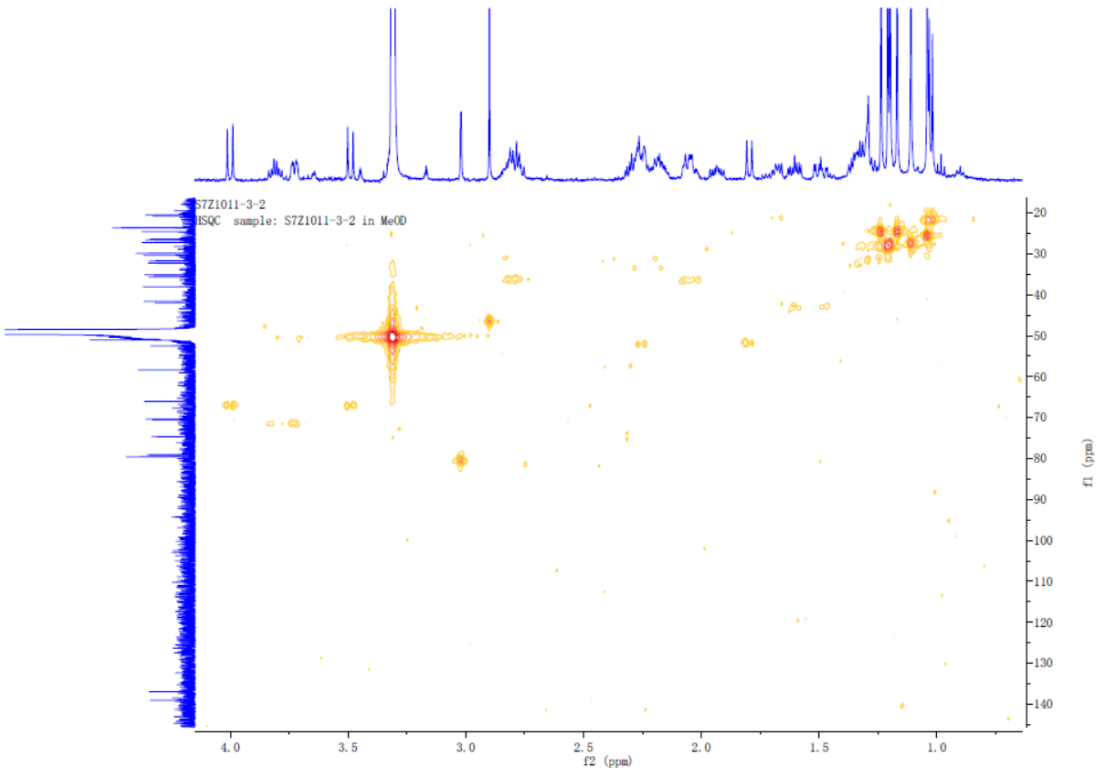

Figure S8. HSQC spectrum of **1** (600 MHz, MeOH-d_4)

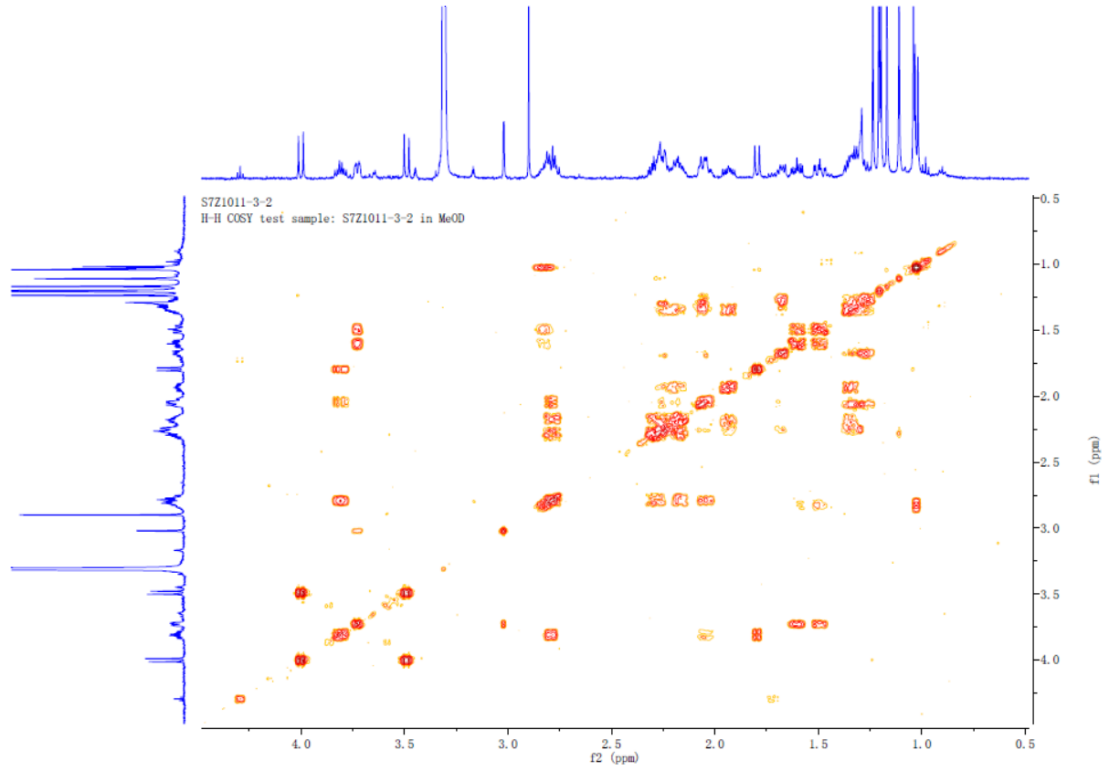

Figure S9. COSY spectrum of **1** (600 MHz, MeOH-d_4)

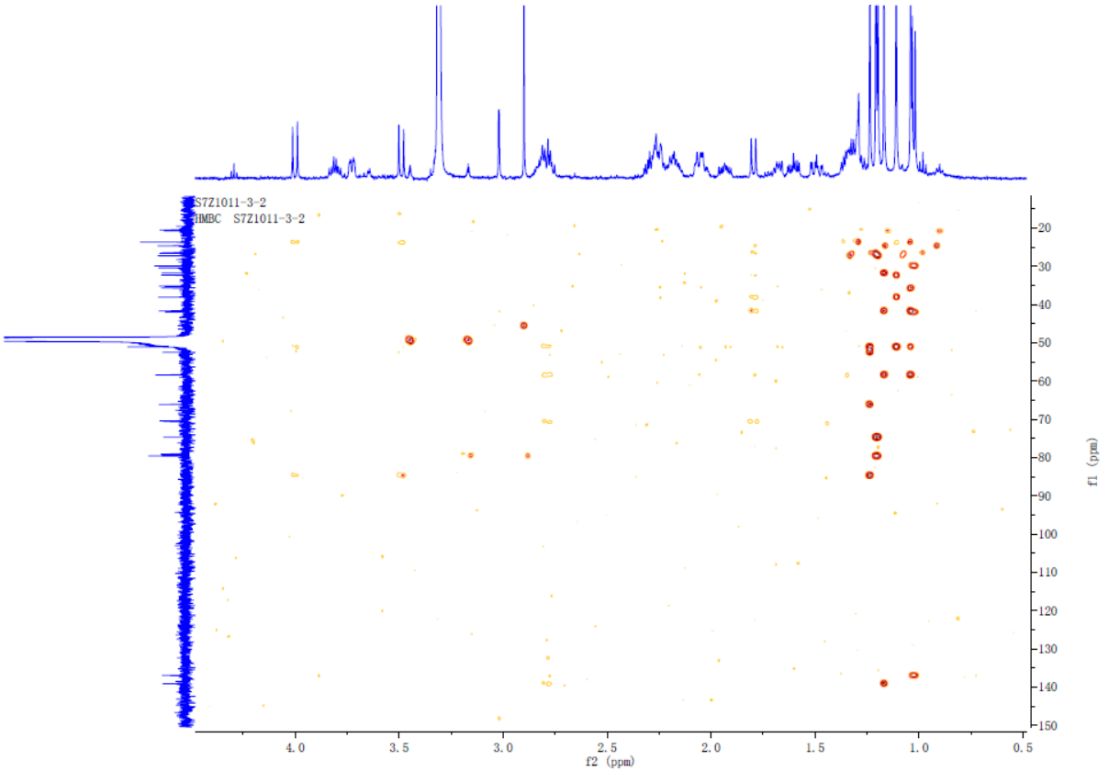

Figure S10. HMBC spectrum of **1** (600 MHz, MeOH-d_4)

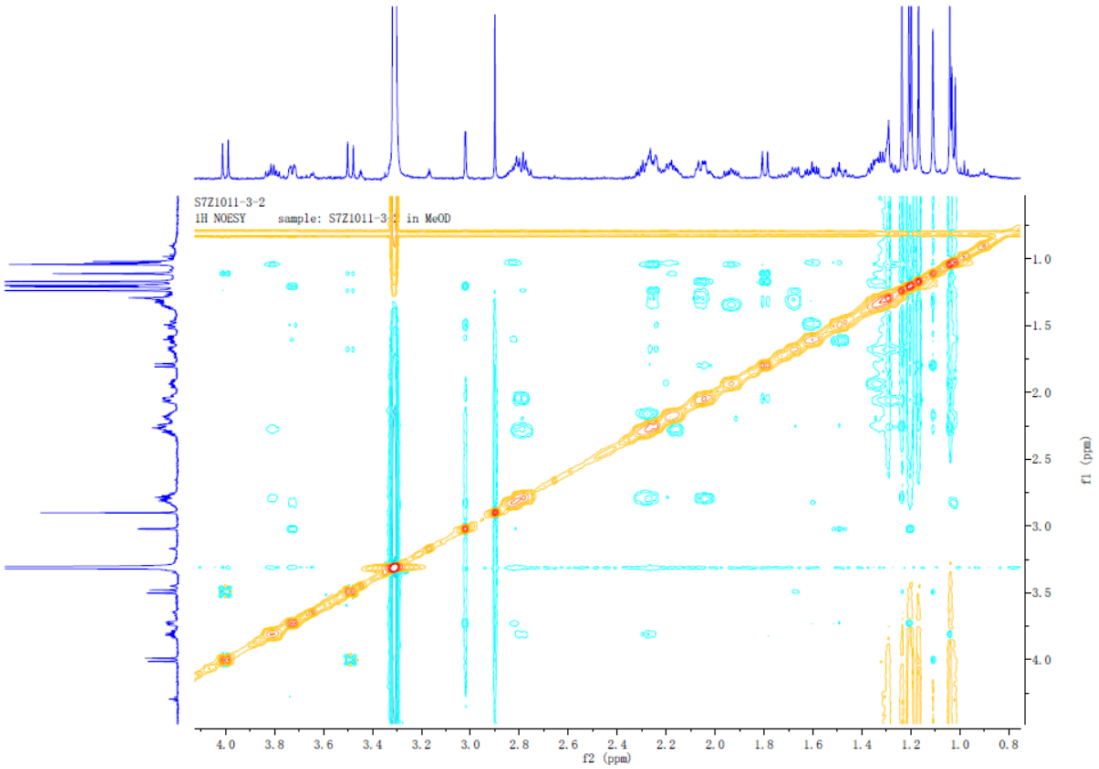

Figure S11. NOESY spectrum of **1** (600 MHz, MeOH-d_4)

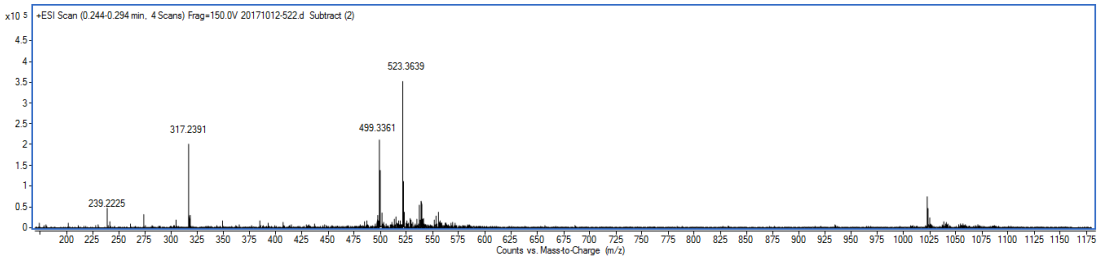

Figure S12. HRESIMS spectrum of **1**